

To Be Mailed 10/790,069



**PCT**

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C07H 15/00</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 99/07720</b> <b>(43) International Publication Date:</b> 18 February 1999 (18.02.99)
<b>(21) International Application Number:</b> PCT/IB98/01188 <b>(22) International Filing Date:</b> 3 August 1998 (03.08.98)  <b>(30) Priority Data:</b> MI97A001880 5 August 1997 (05.08.97) IT  <b>(71) Applicant (for all designated States except US):</b> UNIVERSITY OF MASSACHUSETTS LOWELL [US/US]; One University Avenue, Lowell, MA 01854 (US).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> LAWTON, Carl, William [US/US]; One University Avenue, Lowell, MA 01854 (US). SWARTZ, Randall, Wolfe [US/US]; One University Avenue, Lowell, MA 01854 (US).  <b>(74) Agent:</b> CRAWFORD, Arthur R.; Nixon & Vanderhye P.C., 8th floor, 1100 North Glebe Road, Arlington, Virginia 22201-4714 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> A PROCESS FOR THE PURIFICATION OF ACARBOSE  <b>(57) Abstract</b>  The present invention discloses a process for the purification of acarbose comprising loading a prepurified acarbose solution on a chromatography column packed with a non-aromatic strong acid cation exchanger which is hydrophilic and has high mass transfer and subsequent elution.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

**A PROCESS FOR THE PURIFICATION OF ACARBOSE.**

The present invention relates to a process for the purification of pharmaceutical products, in particular for the purification of acarbose.

**Background of the invention**

5       Acarbose is an inhibitor of the saccharase enzyme complex of human small intestine and is used in medicaments for the treatment of diabetes.

Chemically, acarbose is O-4,6-dideoxy-4-  
10 [(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl       aminoglucopyranosyl-(1->4)-D-glucopyranose.

DE 2209832, DE 2209834 and DE 2064092 disclose the preparation of Acarbose by fermentation of  
15 Actinoplanes species. Dedicated purification processes are disclosed in DE 2347782 and DE 2719912, through the use of strong cation exchangers. These exchangers are gels and macro-reticular type gels, which have poor mass transfer and results in broad peaks upon  
20 elution, therefore lower purities are obtained. The purity resulting from these processes is low, around 77-88% of acarbose in the dry matter (HPLC method).

US 4767850 and US 4666776 disclose the use of strong cation exchanger resins based on hydrophilic  
25 co-monomer and styrene co-monomer.

Strong cation exchangers of the prior art did not assure satisfactory levels of purity. Therefore, it was suggested to use a weakly acid cation exchanger. However, in the prior art there was the opinion that  
30 it was impossible to simply substitute strongly acid

cation exchangers with weakly acid cation exchangers, because the latter can not sufficiently bind acarbose, which is a weak base.

EP 0226121 discloses a process for the  
5 purification of acarbose using a chromatographic column packed with a weakly acid cation exchanger which has carboxyl groups and is based on dextran or agarose or cellulose or exchangers which are derived from the former with the addition of polyacrylamide.  
10 Acarbose purity not lower than 90% by weight, wherein the sugar-like secondary component is present in an amount of less than 10% by weight, but is not completely absent. Examples of up to 98% are given. The above cited patent solved the problem by using a  
15 very special weakly acid cation exchanger, having hydrophilic character and carrying out chromatographic separation in a very narrow pH range.

Use of weak cation exchangers results in higher purity material, however, it is difficult to use on a  
20 production scale because acarbose is not bound but rather slowed in elution. In addition the temperature of the exchanger must be adjusted to optimize the purification.

#### Summary of the invention

25 It has now surprisingly been found that a strong acid cation exchanger of a type different from the ones used till now allows the purification of acarbose on a production scale and with purities of at least 98% .

30 Accordingly, an object of the present invention is a process for the purification of acarbose

comprising contacting an acarbose solution with non-aromatic strong acid cation exchanger, which is hydrophilic and has high mass transfer.

Description of the invention

5       The process for the purification of acarbose essentially comprises:

loading a prepurified acarbose solution in a chromatographic column packed with non-aromatic strong acid cation exchanger, which is hydrophilic and has  
10 high mass transfer; and subsequent elution.

Collecting acarbose-enriched fractions and acarbose isolation are within the common general knowledge of the technician having ordinary skill in the art.

15       To the purpose of carrying out the present invention, prepurified acarbose is prepared by removing impurities coming out from the fermentation process. This operation is generally carried out in two steps:

20       - adsorption: the filtered broth is brought to pH 2.5 with an acid and extracted with active charcoal to remove dark impurities, subsequently pH of the solution is raised to 7.0 with strong anion exchanger in the hydroxide form;

25       - ion exchange: the broth, while keeping pH at 7.0 is contacted with a weak cationic exchanger to lower the conductivity of the broth.

Suitable methods for preparing a prepurified acarbose solution are disclosed in US 4904769 and US  
30 4767850.

The fermentation process to obtain acarbose is

disclosed in US 4062950.

The characterizing part of the process of the present invention is the purification of the prepurified acarbose solution with non-aromatic strong acid cation exchanger, which is hydrophilic and has high mass transfer.

The pH of the prepurified acarbose solution is preferably not lower than 3.0. The solution is then contacted with the exchanger according to the present invention and subsequently eluted with a suitable eluting medium.

~~Ammonia is a preferred example of eluting medium.~~

Other suitable eluting media are, for example, hydrochloric acid, sodium hydroxide or sodium chloride.

The strong cation exchanger according to the present invention is prepared by washing with 1N HCl and then with water until the pH of the effluent is higher than 4.

According to a first preferred embodiment of the present invention, the non-aromatic strong acid cation exchanger, which is hydrophilic and has a high mass transfer, is represented by a polymer-coated alumina matrix. The polymer is obtained from reactive pyridinium monomers and the functional groups are standard propyl sulfonate groups. Strong acid cation exchangers of this type are available on the market under the trademark BioProtocol Bio S by Cohesive Biotechnologies Inc. of Acton Massachusetts.

Alternatively, other strong acid cation exchangers can be used in the process of the present

invention.

In a second preferred embodiment of the present invention, the cation exchanger consists of a sulfoxyethyl cellulose resin, for example Whatman Express-Ion Exchanger, by Whatman.

In a third preferred embodiment of the present invention, the cation exchanger consists of a methacrylate copolymer sulfonate resin, for example Macro-Prep high S, by Bio-Rad.

Finally, the isolation of acarbose may be carried out with conventional techniques, which are well-known to the skilled person. For example, acarbose-enriched fractions can be concentrated to supersaturation, by vacuum-evaporation, then the product of interest is precipitated from a suitable medium, such as acetone.

The purity of acarbose obtainable according to the process of the present invention is at least 98%.

The preparations obtainable by the process according to the present invention, containing acarbose with a content of at least 98% w/w, wherein a secondary component, which is identifiable as a sugar, is present at most in an amount of 2%, and optionally water is contained, are a within the scope of the claims.

Analogously, pharmaceutical compositions, containing a therapeutically effective amount of a preparation as above described, are a further object of the present invention.

Pharmaceutical compositions and related preparations are disclosed in EP 0226121.

A still further object of the present invention

is the use of the above preparations for the manufacture of a medicament having inhibiting activity of the saccharase enzyme complex of human small intestine, useful, for example, for the treatment of diabetes.

The following Examples further describe the present invention.

Example 1

a) Prepurified acarbose

100 ml of a filtered cell free solution coming from the fermentation were adjusted to pH 2.5 with HNO<sub>3</sub>. The mixture was stirred for 10 minutes with 0.5 grams of active charcoal, and then centrifuged for 30 minutes at 5000 rpm. The solution was then neutralized by adding 2.5 grams of Amberlite IRA 410 (OH<sup>-</sup> form). The neutral supernatant liquid was then contacted with 2.5 grams of IRC-50 to lower the conductivity.

Chromatography

b) A chromatography column of 4.6 mm diameter and 100 mm length (1.7 ml volume) was packed with strong cation resin (BioProtocol Bio S) in distilled water. The column was washed with 10 ml column volumes of 0.01 N HCl followed by 10 column volumes of distilled water at a flux of 3 ml/min. 800 µl of the solution of prepurified acarbose, as prepared according to item a) above, were injected in the column. The column was eluted with 10 column volumes of 0.2 N NH<sub>4</sub>OH. The eluate was collected and analyzed by HPLC (as described in US 4904769) and its purity was 98%



in the dry matter.

Example 2

The method of example 1 was repeated except the strong acid cation exchanger was Whatman  
5 Sulfoethoxyethyl (SE) Cellulose Express-Ion Exchanger.

Example 3

The method of example 1 was repeated except the strong acid cation exchanger was Macro-Prep high S, by Bio-Rad.

Claims

1. A process for the purification of acarbose comprising loading a prepurified acarbose solution on a chromatography column packed with a non-aromatic strong acid cation exchanger which is hydrophilic and has high mass transfer and subsequent elution.
2. A process according to claim 1, wherein said strong acid cation exchanger is based on alumina particles coated with a polymer, said polymer being obtained from reactive pyridinium monomers and bearing propyl sulfonate functional groups.
3. A process according to claim 1, wherein said strong acid cation exchanger is based on sulfoxyethyl cellulose resin.
4. A process according to claim 1, wherein said strong acid cation exchanger is based on methacrylate copolymer sulfonate.
5. A process according to anyone of claims 1-4, wherein said elution is carried out with ammonia.
6. A process according to anyone of claims 1-4, wherein said elution is carried out with sodium hydroxide.
7. A process according to anyone of claims 1-4, wherein said elution is carried out with hydrochloric acid.
8. A preparation containing acarbose with a content of not less than 98% by weight, a secondary sugar-like component at most 2%, optionally water, obtainable by the process of anyone of

claims 1-7.

9 A pharmaceutical composition containing as active ingredient an effective amount of a composition of claim 8.

5 10 The use of a preparation of claim 8 for the preparation of a medicament having inhibiting activity of the saccharase enzyme complex of human small intestine.

11 The use according to claim 10, wherein the  
10 medicament is useful for the treatment of diabetes.